DP-MS-78-71

ISOLATION OF *NAEGLERIA FOWLERI* FROM ARTIFICIALLY HEATED WATER

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A paper proposed for publication in *Nature* as a letter.

This paper was prepared in connection with work under Contract No. AT(07-2)-1 with the U.S. Department of Energy.

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DISCLAIMER

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Power generating systems in the United States will probably reach 1.3×10^6 megawatts by 1990 requiring an additional 7.5×10^6 gpm of cooling water.[1] The discharge of any waste heat from industralization into southeastern aquatic habitats results in the heating of already warm water, 25-35°C.[2,3] To determine if such thermal discharges into warm waters enhance the isolation frequency of *Naegleria fowleri*, we compared a variety of aquatic habitats altered by man-made thermal discharges at the Savannah River Plant (SRP), near Aiken, South Carolina. Additionally, control samples were collected from ambient temperature lakes, ponds, the Savannah River, and the unheated artificial streams at the Flowing Streams Laboratory (FSL).[4] Of the 79

* The information contained in this article was developed during the course of work under Contract No. AT(07-2)-1 with the U.S. Department of Energy.

samples from artificially heated habitats, 55 demonstrated outgrowth at 45°C and 34 had flagellated amoebae characteristic of *N. fowleri.*[5] Only one high temperature flagellated amoeba was isolated from the 51 samples collected from ambient temperature habitats. Our data indicate that *N. fowleri* is more frequently isolated from thermally altered habitats, and that the isolation frequency increase where habitat temperatures are between 40° and 60°C.

Selected free-living species of *Acanthamoeba* and *Naegleria* (*N. fowleri*) are causative agents of primary amoebic meningoencephalitis (PAM).[6,7] The true incidence of the disease is probably unknown because many cases are either unrecognized or incorrectly diagnosed; however over 150 cases of fatal PAM have been attributed to *N. fowleri* and *Acanthamoeba*. Subsequent epidemiological studies indicated that many cases of PAM occurred in individuals who had been in close contact with heated water.[8,9]

Griffin[10] demonstrated that *N. fowleri* and *A. culbertsoni* grew *in vitro* at temperatures above 37°C while their nonpathogenic counterparts did not, and suggested that thermal additions to aquatic systems may select for pathogenic species of the ubiquitous free-living amoebae. De Jonckheere, et al.[5] noted that in Belgium, *N. fowleri* has been isolated only from artificially heated water. Recent studies in the U.S. also demonstrated that *N. fowleri* isolated more frequently from southeastern aquatic systems during the summer months.[2,11,12]

In this study, water, sediment, and algal samples were collected from both thermal and ambient temperature systems throughout the 777 square kilometer (SRP) site. The majority of samples were collected from either the Par Pond system[3] or from artificially heated streams at the FSL.[4] The Par Pond system consists of a 1092 hectare cooling reservoir which is a source of 90% of the water used to cool a production reactor. The resulting thermal effluent (ca 768,000 1/min) is discharged to the environment through a series of canals and cooling ponds, 11 kilometers long, before being discharged again into Par Pond. Within the discharge canal system, thermal gradients are established with corresponding algal and bacterial communities. The second major habitat, FSL, is a greenhouse facility containing four artificially heated streams. Water from an unheated blackwater stream, Upper Three Runs Creek, is heated to a temperature of 25°C above ambient and then mixed with increasing amounts of ambient temperature water to achieve streams of 12.5°, 7.5°, 5.0°, and 2.5°C above ambient. Additionally, two control streams are maintained with ambient temperature water.

All 130 samples were collected aseptically and processed immediately in the field or within 24 hours after returning to the laboratory. Temperature, pH, and dissolved oxygen were measured at the time of collection. Water samples were vacuum filtered through sterile cellulose acetate membrane filters (1.2 nm) at 25.4 cm of mercury. The filters were removed, aseptically cut

in half, and inverted onto nonnutrient agar plates seeded with heat-killed and unheated *Escherichia coli*. Algal communities, microbial mats, and sediments were placed directly on *E. coli* seeded plates. Plates were sealed with parafilm, incubated at 45°C, and observed daily for outgrowth. When sufficient outgrowth had occurred, amoebae were harvested by washing with sterile water and transferred to newly prepared *E. coli* seeded plates. Stock cultures were maintained on *E. coli* seeded plates or axenically in a casitone-based medium.[13]

Amoebic isolates were identified as *N. fowleri* on the basis of their ability to grow at 45°C, their typical trophozoite and cyst morphology, and their ability to flagellate. Additionally, serological analyses were made using indirect immunofluorescence with antisera prepared against the pathogen *N. fowleri*[5] and the nonpathogenic *Naegleria*, i.e., *N. jadini* and *N. gruberi*. The isolates reacted maximally with antisera against pathogenic *N. fowleri* (Morgan), although minimal reaction was noted with antisera against *N. jadini* and *N. gruberi*. Pathogenicity of the isolates was tested by intranasal inoculation of 8-16 $\times$ 10^4 trophozoites into ether-anesthetized 4-6 week ICR or BALB/c mice. Some isolates produced fatal meningoencephalitis on intranasal inoculation and were readily reisolated from infected brain tissue, while other isolates produced no PAM. Serological analyses of selected nonpathogenic isolates confirmed them as *N. fowleri*, and are considered as nonpathogenic variants often associated with artificially heated water.[5,14]

The data for studies conducted from January to May of 1978 are summarized in Table 1, and indicate that 43% of the samples taken from thermally altered habitats were positive for *N. fowleri* while ambient temperature samples were positive only 1.9% of the time. The data further indicate that *N. fowleri* are more frequently isolated from habitats with elevated temperatures than cooler habitats (Figure 1). It is interesting to note that the heated streams at FSL had been operating only about 18 months when the first isolation of *N. fowleri* was made, while control streams have continued negative by our sampling techniques. This suggests that *N. fowleri* can colonize a thermally altered habitat rather quickly. Additionally, thermal systems at the site have existed since the early 1950's; thus, the presence of *N. fowleri* in these systems is probably not recent.

Our studies suggest that thermally altered aquatic systems may provide habitats that are conducive to the growth and proliferation of *N. fowleri*. The ecology of pathogenic micro-organisms associated with the receiving waters merits further study.

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TABLE 1

Summary Data for the Presence of Thermophilic Flagellated Amoeba at the Savannah River Plant

Habitat	*Temperature, °C*	*Number of Samples*	*Samples With Outgrowth of Amoeba at 45°C*	*Samples With Flagellated Amoeba*	*Number of Flagellated Amoeba/ Number of Samples*
Artificially heated	9.8 - 71.5	79	55	34	43.0
Ambient temperature	5.2 - 28.5	51	7	1	1.9

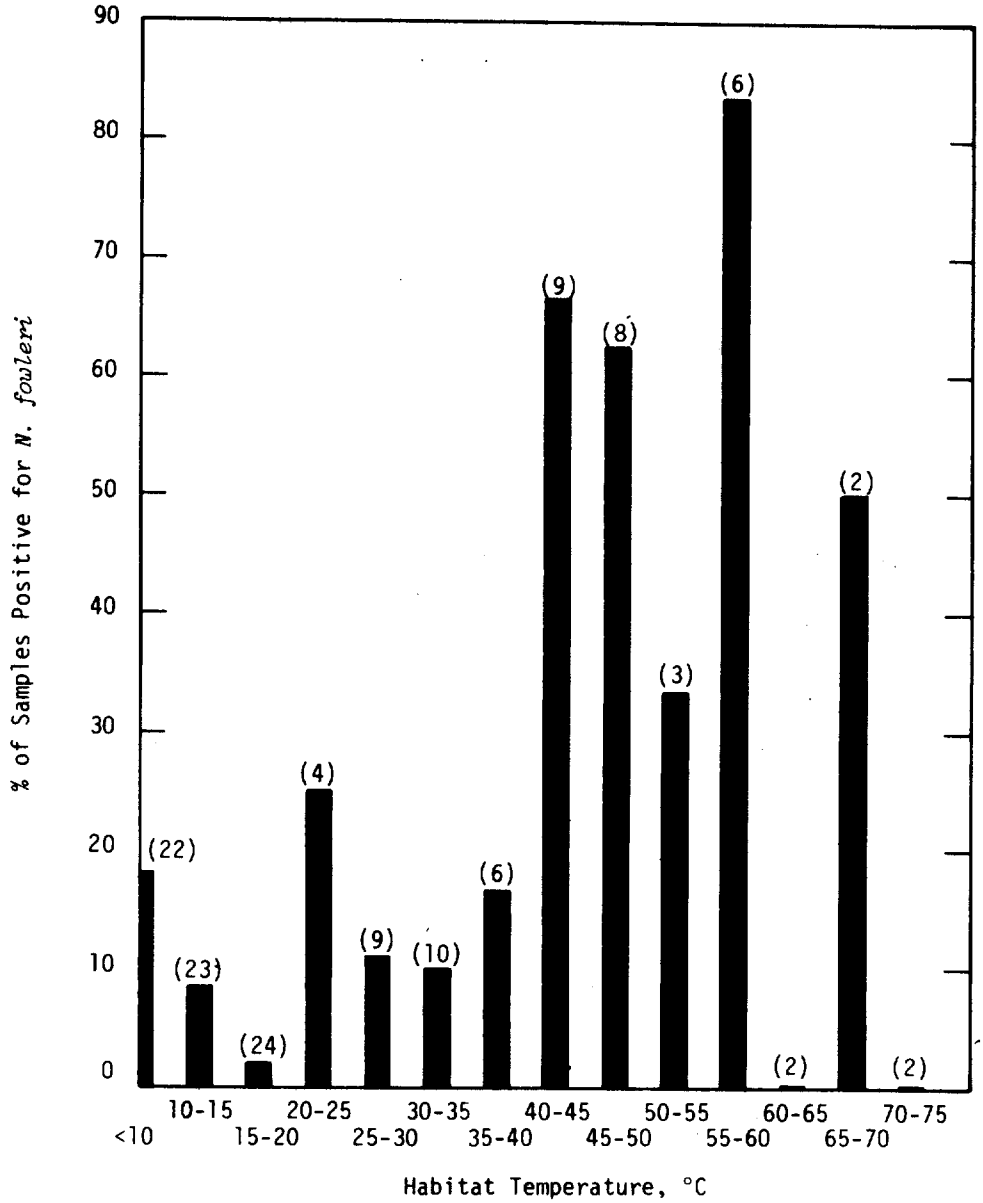

FIGURE 1. Percentage of Samples Positive for *N. fowleri* with Respect to Habitat Temperature (sample size in parentheses)